

Amendments to the Claims:

This listing of claims will replace, without prejudice, all prior versions, and listings, of claim in this application.

Listing of Claims:

1. *(Original)* A method of crystallizing a macrolide from a macrolide starting material comprising the steps of:
 - a) combining a macrolide starting material, a polar solvent, a hydrocarbon solvent, and water, whereby at least two phases are formed, at least one of which is a water-rich phase, and wherein the pH of the water-rich phase is at least about 7,
 - b) maintaining the combination at for at least 1 hour, whereby a macrolide-rich phase is formed from which the macrolide crystallizes.
2. *(Original)* The method of claim 1 further comprising the step of isolating the macrolide that crystallizes.
3. *(Original)* The method of claim 1 wherein the combination of step b is maintained at a temperature of from about -15°C to about 50°C.
4. *(Original)* The method of claim 3 wherein the combination of step b is maintained at a temperature of from about -5°C to about 40°C.
5. *(Original)* The method of claim 4 wherein the combination of step b is maintained at a temperature of from about -2°C and about 35°C.
6. *(Original)* The method of claim 1 wherein the combination of step b is maintained for between 48 and 100 hours.

7. *(Original)* The method of claim 1 wherein the polar solvent is selected from the group consisting of alcohols, esters, nitriles and ethers.
8. *(Original)* The method of claim 7 wherein the polar solvent is selected from the group consisting of ethyl acetate, acetonitrile, methanol, ethanol, *n*-propanol, *iso*-propanol, *n*-butanol, *iso*-butanol, acetone, diisopropyl ether, dimethyl formamide, and dimethyl acetamide.
9. *(Original)* The method of claim 8 wherein the polar solvent is ethyl acetate.
10. *(Original)* The method of claim 1 wherein the hydrocarbon solvent is selected from the group consisting of *n*-hexane, *n*-heptane, octane, *iso*-octane cyclohexane, methylcyclohexane, benzene, toluene, and xylene.
11. *(Original)* The method of claim 10 wherein the hydrocarbon solvent is *n*-hexane.
12. *(Original)* The method of claim 1 wherein the pH of the water-rich phase is about 8 or higher.
13. *(Original)* The method of claim 1 wherein the water comprises a base selected from NaOH, KOH, Ca(OH)₂, NH₃, Et₃N, diethylamine and pyridine.
14. *(Currently Amended)* The method of claim 1 wherein the macrolide is selected from the group consisting of tacrolimus, sirolimus, pimecrolimus, and everolimus, and ascomycin.
15. *(Original)* A method of crystallizing a macrolide from a macrolide starting material comprising the steps of:
 - a) combining a concentrate residue from whole-broth extraction of macrolide-containing biomatter in a polar solvent with a hydrocarbon solvent, and water, whereby at

least two phases are formed, at least one of which is a water-rich phase, and wherein the pH of the water-rich phase is at least about 7,

b) maintaining the combination at for at least 1 hour, whereby a macrolide-rich phase is formed from which the macrolide crystallizes.

16. *(Original)* The method of claim 15 further comprising the step of isolating the macrolide that crystallizes.

17. *(Original)* The method of claim 15 wherein the combination of step b is maintained at a temperature of from about -15°C to about 50°C.

18. *(Original)* The method of claim 17 wherein the combination of step b is maintained at a temperature of from about -5°C to about 40°C.

19. *(Original)* The method of claim 18 wherein the combination of step b is maintained at a temperature of from about -2°C and about 35°C.

20. *(Currently amended)* The method of claim 15 wherein the combination of step b is maintained for between 48 and 100 hours.

21. *(Original)* The method of claim 15 wherein the polar solvent is selected from the group consisting of alcohols, esters, nitriles and ethers.

22. *(Original)* The method of claim 21 wherein the polar solvent is selected from the group consisting of ethyl acetate, acetonitrile, methanol, ethanol, *n*-propanol, *iso*-propanol, *n*-butanol, *iso*-butanol, acetone, diisopropyl ether, dimethyl formamide, and dimethyl acetamide.

23. *(Original)* The method of claim 22 wherein the polar solvent is ethyl acetate.

24. *(Original)* The method of claim 15 wherein the hydrocarbon solvent is selected from the group consisting of *n*-hexane, *n*-heptane, octane, *iso*-octane cyclohexane, methylcyclohexane, benzene, toluene, and xylene.
25. *(Original)* The method of claim 24 wherein the hydrocarbon solvent is *n*-hexane.
26. *(Original)* The method of claim 15 wherein the pH of the water-rich phase is about 8 or higher.
27. *(Original)* The method of claim 15 wherein the water comprises a base selected from NaOH, KOH, Ca(OH)₂, NH₃, Et₃N, diethylamine and pyridine.
28. *(Currently amended)* The method of claim 15 wherein the macrolide is selected from the group consisting of tacrolimus, sirolimus, pimecrolimus, and everolimus, and ascomycin.
29. *(Original)* A method of crystallizing a macrolide from a macrolide starting material comprising the steps of:
 - a) combining, at a temperature of about 20° to about 25°C, macrolide starting material, ethyl acetate, *n*-hexane, and a water solution of a base selected from NaOH, KOH, Ca(OH)₂, NH₃, (C₂H₅)₃N, diethylamine and pyridine whereby at least two phases are formed, one of which is a water-rich phase, wherein the pH of the water-rich phase is > about 7,
 - b) maintaining the combination at a temperature of about 20°C to about 25°C for at least 1 hour, whereby a macrolide-rich phase is formed from which macrolide crystallizes,
 - c) maintaining the combination at a temperature of about 0°C to about 20°C for at least 1 hour, and
 - d) recovering the macrolide that crystallizes.

30. (*Currently amended*) The method of claim 29 wherein the macrolide is selected from the group consisting of tacrolimus, sirolimus, pimecrolimus, and everolimus, and ascomycin.

31. (*Original*) The method of claim 29 wherein the pH of the water-rich phase is about 8 or higher.

32. (*Original*) A method of crystallizing a macrolide from a macrolide starting material comprising the steps of:

a) combining, at a temperature of about 20° to about 25°C, a concentrate residue from whole-broth extraction of macrolide-containing biomatter in ethyl acetate, *n*-hexane, and a water solution of a base selected from NaOH, KOH, Ca(OH)₂, NH₃, (C₂H₅)₃N, diethylamine and pyridine whereby at least two phases are formed, one of which is a water-rich phase, wherein the pH of the water-rich phase is > about 7,

b) maintaining the combination at a temperature of about 20°C to about 25°C for at least 1 hour, whereby a macrolide-rich phase is formed from which macrolide crystallizes,

c) maintaining the combination at a temperature of about 0°C to about 20°C for at least 1 hour, and

d) recovering the macrolide that crystallizes.

33. (*Currently amended*) The method of claim 32 wherein the macrolide is selected from the group consisting of tacrolimus, sirolimus, pimecrolimus, and everolimus, and ascomycin.

34. (*Original*) The method of claim 32 wherein the pH of the water-rich phase is about 8 or higher.

35. (*Original*) In a method for crystallizing a macrolide from a macrolide starting material, the step of combining the macrolide starting material, a polar solvent, a

hydrocarbon solvent, and water, whereby at least two phases are formed, at least one of which is water rich, wherein the pH of the water-rich phase is at least about 7.

36. *(Original)* In a method for crystallizing a macrolide from a concentrate residue from whole-broth extraction of macrolide-containing biomatter in a polar solvent, the step of combining the macrolide concentrate in the polar solvent, a hydrocarbon solvent, and water, whereby at least two phases are formed, at least one of which is water rich, wherein the pH of the water-rich phase is at least about 7.